

Microbial Transformation Kinetics of
Organic Compounds

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16. ABSTRACT One process determining the fate of xenobiotics (organics not natural to a system) in environmental waters is bacterial transformation. Bacteria are one of the most metabolically active groups of organisms in the environment. They can degrade and derive energy from a variety of organic compounds. Increases in the number of synthetic compounds and their accumulation in the environment have led to a more quantitative and critical view of microbial metabolism of xenobiotics. Rates of microbial degradation depend upon both the structure of the compound and on the metabolic activity of the bacteria in the ecosystem receiving the xenobiotic. Studies of second-order kinetics are a useful beginning in the detailed quantitative study of rates of biotransformation of xenobiotics. Many questions about the role of temperature, other nutrients, population structure, and compound structure are still unanswered. Only additional kinetic studies for many compounds and sites can provide confidence in the approach and the ultimate answers to these questions.		
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Microbial Transformation Kinetics of Organic Compounds

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Introduction

One process determining the fate of xenobiotics (organics not natural to a system) in environmental waters is bacterial transformation. Bacteria are one of the most metabolically active groups of organisms in the environment. They can degrade and derive energy from a variety of organic compounds. This characteristic probably fostered the idea that bacteria are infallible and can degrade any organic compound. Accumulation of compounds like DDT and its products (DDD and DDE) throughout the food chain indicate, however, that not all organic compounds are readily degraded. Those not degraded to a significant extent have been termed "recalcitrant" [1]. To identify recalcitrant organics, the surfactant test [6, 24], the sludge test [5], and other qualitative tests for degradability are used almost routinely in detergent manufacturing and other chemical industries.

Increases in the number of synthetic compounds and their accumulation in the environment have led to a more quantitative and critical view of microbial metabolism of xenobiotics. No longer is it sufficient to know only whether or not a compound is biodegradable. Instead rates, products, microbial populations and environmental conditions that relate to the biodegradation process are of interest to microbiologists and ecologists. Rates of microbial degradation depend upon both the structure of the compound and on the metabolic activity of the bacteria in the ecosystem receiving the xenobiotic.

Kinetics of Degradation of Xenobiotics by Mixed Microbial Populations

Microbial communities derive energy for metabolism and growth from the degradation of organic compounds. The kinetics of growth and substrate utilization have been studied by microbiologists for many years [9, 18, 23, 29]. Monod [13]

showed that the growth rate of a microorganism in pure culture on a growth limiting substrate can be described by the equation

$$\frac{d[B]}{dt} = \mu[B], \quad (1)$$

where: $[B]$ is the biomass (cells or mass/liter) and μ is the specific growth rate (h^{-1}).

The relationship between growth rate of bacteria and concentration of a limiting substrate can be described by

$$\mu = \frac{\mu_{\max}[S]}{K_s + [S]}, \quad (2)$$

where: K_s is the saturation constant or substrate concentration (mg/l) at one-half $\mu_{\max}$ and $\mu_{\max}$ is the maximum specific growth rate (h^{-1}). Substituting Eq. 2 in 1 yields:

$$\frac{d[B]}{dt} = \frac{\mu_{\max}[S][B]}{K_s + [S]}. \quad (3)$$

Assuming a fixed amount of growth results from metabolism of a unit quantity of substrate

$$d[B] = -Yd[S], \quad (4)$$

where: Y is the yield coefficient in cells or mass produced per unit of substrate metabolized. Y describes the efficiency with which the substrate is converted to biomass. To obtain the substrate transformation rate, Eq. 3 is substituted in Eq. 4 and rearranged to yield:

$$-\frac{d[S]}{dt} = \frac{\mu_{\max}[B][S]}{Y(K_s + [S])}. \quad (5)$$

The observed microbial growth yield is often calculated from the amount of microbial biomass produced during the course of transformation of a measured quantity of substrate. Because a microbial population must satisfy maintenance requirements before any growth can occur, the apparent yield can fall quite drastically at low concentrations of S . Expressions having separate growth and maintenance yields have been developed by Pirt [17] and it is possible to compute the growth and maintenance yields from microbial growth experiments [20].

The Monod formulation (Eq. 5) has been successfully applied to biotransformation of xenobiotics in the laboratory [12, 14, 15, 21]. These studies have demonstrated that the Monod equation can adequately describe substrate transformations by heterogeneous bacterial populations as well as single-species populations [10]. In one example, Paris, Lewis, and Wolfe [15] isolated an assemblage of four genus-different bacteria that were able to use malathion as their sole source of carbon. As bacterial numbers increased, malathion decreased (Fig. 1) [14]. Analysis of the growth response of the population and of concurrent malathion transformation rates then gave the Monod parameters $\mu_{\max} = 0.37 \text{ h}^{-1}$, $K_s = 2.17 \text{ } \mu\text{M/liter}$ (0.716 mg/liter), and $Y = 4.1 \times 10^{10} \text{ cells/} \mu\text{M}$ ($1.2 \times 10^{11} \text{ cells/mg}$). In a similar study, Smith and co-workers [21] investigated the degradation of *p*-cresol by mixed microbial cultures able to use the compound as a sole carbon source. These authors

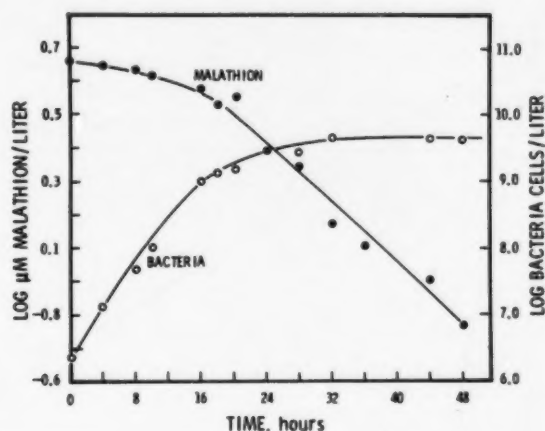


Fig. 1. Growth of bacteria and decrease in malathion concentration

expressed their results in terms of Monod parameters, finding $\mu_m = 0.62 \text{ h}^{-1}$, $Y = 1.9 \times 10^9 \text{ cells/mg}$ and $K_s = 0.84 \text{ mg/liter}$.

Unfortunately, applications of such classical microbiological methods to biotransformation of xenobiotics are difficult to apply in a broader ecological context. One difficulty is that the Monod formulation (Eq. 5) is non-linear in its parameters. However, for trace concentrations of xenobiotics (i.e., $[S] \ll K_s$) that would occur in the environment the term $(K_s + [S])$ can be approximated by K_s , giving the linear approximation

$$\frac{d[S]}{dt} = - \frac{\mu_m [B][S]}{YK_s} \quad (6)$$

This equation is similar to the "second-order" equations used to describe the kinetics of chemical reactions, and the term (μ_m/YK_s) can be termed, by analogy, a second-order biotransformation rate constant, k_{b2} , with units of $\text{liter cell}^{-1} \text{ hour}^{-1}$.

From values given above for malathion, $\mu_m/YK_s = 0.37/(4.1 \times 10^{10})(2.17) = 4.16 \times 10^{-12} \text{ liter cell}^{-1} \text{ h}^{-1}$. Paris et al. [15] also computed values of k_{b2} from microbial population size and malathion transformation rates in their experimental studies, finding a mean value for k_{b2} of $(2.6 \pm 0.7) \times 10^{-12}$ (95% confidence interval) $\text{liter cell}^{-1} \text{ h}^{-1}$ for a series of eight experimental determinations spanning a concentration range from 0.0273 to 0.33 μM in malathion. The measured k_{b2} differed from μ_m/YK_s by less than a factor of 2, suggesting that simplified kinetic experiments could be used to develop second-order rate constants, when the concentration of the compound is $\ll K_s$.

Furthermore, dissolved pesticide concentrations in surface waters are often very low. For example, dieldrin, lindane, and DDT have been found primarily at part per trillion levels both in the USA [11] and in Britain [4]. For industrial chemicals, however, the situation can be somewhat different. Visser et al. [26] report release of phenol-containing wastes into the St. Lawrence River resulting in concen-

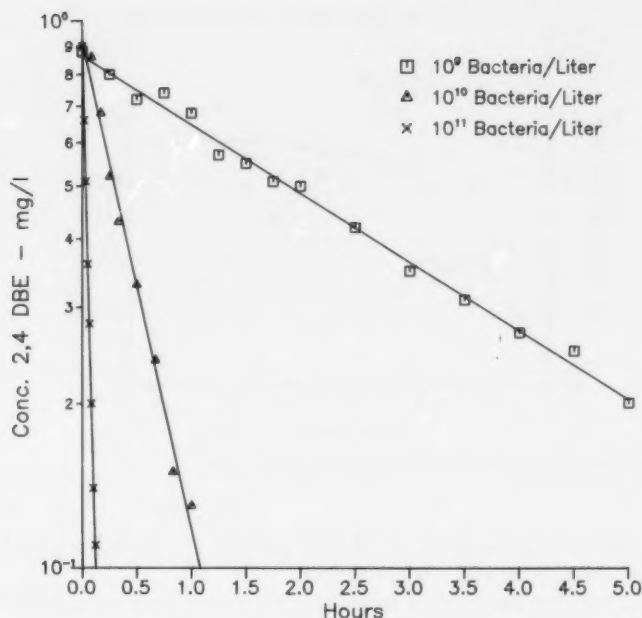


Fig. 2. Pseudo-first-order plot for microbial degradation of butoxy ethyl ester of 2,4-dichlorophenoxyacetic acid with three bacterial concentrations

trations of 10 to 150 ppb. Although these high concentrations are restricted to a dispersion cone immediately downstream of the effluents, K_s for *p*-cresol (840 ppb) is close to the highest measured concentrations.

An evaluation of the suitability of Eqs. 4 and 5 for environmental situations presents some difficulties. A natural microbial community derives its energy from a large variety of organic detrital materials. Even when a synthetic compound is sufficiently similar to natural substrates that it can be degraded by the microbial community as a whole, the presence of multiple energy-yielding substrates in the environment violates a fundamental assumption underlying the Monod approach, that is, that $[S]$, the synthetic compound, limits growth. In many instances, moreover, a compound may be transformed in an energy-requiring detoxification process, making the concept of cell yield (Y) of little use. When the compound is degraded in the absence of a change in population size, the apparent zero yield forces abandonment of Eqs. 4 and 5.

Despite these difficulties, the rate of transformation of xenobiotics must depend on the structure of the compound and the metabolic potential of microbial communities. The simplest expression of this duality is the second-order equation:

$$-\frac{d[S]}{dt} = k_{b2}[B][S], \quad (7)$$

Table 1. Second-order degradation rate constants for three compounds in water

Compound	k_{b2} (l org ⁻¹ h ⁻¹) ^a	N^b
2,4-D butoxyethyl ester	$(5.42 \pm 0.97) \times 10^{-10}$	31
Malathion	$(4.54 \pm 0.74) \times 10^{-11}$	14
Chlorpropham	$(2.63 \pm 0.72) \times 10^{-14}$	11

^a 95 percent confidence intervals based on between site variation^b Number of sites for which k_{b2} was determined

which asserts that the rate of microbial transformation of substrate ($d[S]/dt$) is first-order in substrate concentration $[S]$ and in microbial concentration $[B]$ (Fig. 2). This approach requires demonstration that $d[S]/dt$ is linearly proportional to $[S]$ for fixed levels of B and that appropriate measurements of B be used.

One conventional microbiological technique for characterizing microbial populations is population size, measured, for example, by colony counts on pour-plate agar [2]. To the extent that population size is an adequate measure of community metabolic capacity, simple microbiological techniques could serve to characterize the rate of biodegradation of xenobiotics in natural environments. This hypothesis has been explored for three compounds [butoxyethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-DBE), malathion, and chlorpropham] by Paris and co-workers [3, 16]. These authors investigated biotransformation of the compounds studied in samples of natural waters from 40 locations in the continental United States. Ambient water temperatures at the collection points ranged from 1° to 29 °C, and the laboratory studies were conducted at the observed ambient temperature of the sampled environments. Bacterial populations as measured by 48-h incubation on tryptone glucose extract agar at 22 °C, ranged from 4.0×10^5 to 9.0×10^8 cells/liter [15]. Transformation of these chemicals was demonstrably first-order in compound concentration and in population size, and was relatively independent of temperature. Second order degradation rate constants for these three compounds are shown in Table 1.

Evaluation of microbial transformations in such "river die-away" studies is difficult when a combination of small populations and slow rates of biodegradation leads to long biolysis half-lives. In such cases population densities must be augmented via centrifugation or supplemental nutrient broth. The latter procedure can have the disadvantage of favoring opportunistic heterotrophs at the expense of organisms better equipped to degrade xenobiotics. For example, the second order microbial transformation rate constant (k_{b2}) for chlorpropham was found to be unaffected when population densities were augmented via nutrient broth supplementation, but the measured k_{b2} for phenanthrene decreased in approximate proportion with the population increase (unpublished data).

Baughman, Paris, and Steen [3] also reported second order rate constant for biotransformation of xenobiotics by populations derived from aquatic sediments. For suspended aerobic sediment systems, plate counts could not be used and it was necessary to use adenosine triphosphate (ATP) measurement. The total ATP in the

Table 2. Rate constants for transformation in sediment-water systems

Compound	Fraction sorbed	k_{app} (1 org ⁻¹ h ⁻¹ ^d)	k_2 (1 org ⁻¹ h ⁻¹ ^d)
2,4-D butoxyethyl ester ^a	0 ^b	$(2.3 \pm 0.8) \times 10^{-10}$	
Malathion ^a	0 ^b	$(4.0 \pm 0.9) \times 10^{-11}$	
Chlorpropham ^c	0 ^b	$(1.42 \pm 0.6) \times 10^{-13}$	
	0.15	$(4.15 \pm 0.7) \times 10^{-13}$	$(3.45 \pm 0.2) \times 10^{-13}$
	0.90	$(7.4 \pm 1.2) \times 10^{-15}$	$(2.29 \pm 0.6) \times 10^{-13}$
Methoxychlor ^a	0 ^b	$(6.7 \pm 1.2) \times 10^{-14}$	
	0.15	$(5.2 \pm 2.3) \times 10^{-14}$	$(3.0 \pm 0.5) \times 10^{-14}$
	0.90	$(6.1 \pm 2.8) \times 10^{-16}$	$(3.2 \pm 1.2) \times 10^{-14}$
<i>bis</i> (2-Ethylhexyl)-phthalate ^a	0 ^b	$(4.2 \pm 0.7) \times 10^{-15}$	
	0.15	$(1.8 \pm 0.3) \times 10^{-15}$	$(3.6 \pm 1.3) \times 10^{-15}$
	0.90	$(5.7 \pm 1.7) \times 10^{-17}$	$(6.1 \pm 2.2) \times 10^{-15}$
Dibutylphthalate ^a	0 ^b	$(3.1 \pm 0.8) \times 10^{-11}$	
	0.15	$(2.7 \pm 1.5) \times 10^{-11}$	$(3.5 \pm 1.7) \times 10^{-11}$
	0.90	$(6.1 \pm 0.5) \times 10^{-13}$	$(2.6 \pm 0.2) \times 10^{-11}$
<i>Di</i> (<i>n</i> -octyl)-phthalate ^a	0 ^b	$(3.7 \pm 0.6) \times 10^{-13}$	
	0.15	$(2.1 \pm 1.2) \times 10^{-13}$	$(3.6 \pm 0.7) \times 10^{-13}$
	0.90	$(3.1 \pm 0.4) \times 10^{-15}$	$(6.9 \pm 2.3) \times 10^{-13}$

^a Data from Steen in preparation^b Sediment free^c Data from Steen et al. [22]^d mean $\pm$ standard deviation for 4 determinations

system was used as a direct measurement of the mixed microbial population size by comparison with plate counts and ATP for water alone. Inherent in these tests was the incorporation of sediment sorption effects on the observed second-order expression. Sediment sorption was treated by addressing distribution of xenobiotics between sorbed and dissolved forms the sum of which is the total substrate in the system. Table 2 shows the rate constants obtained via Eq. 8:

$$-\frac{d[S]_T}{dt} = \frac{k_{b2}}{1 + \rho K_p} [B][S]_T, \quad (8)$$

where: k_{b2} is the second-order rate constant from Eq. 7, ρ is the sediment-to-water-mass ratio, K_p is the partition coefficient of substrate between sediment and water, k_{app} is the apparent second-order rate constant, and $[S]_T$ is the total amount of substrate per unit aqueous volume of the system.

The observed rate constants for 2,4-DBE and malathion were consistent with the rate of degradation by water-column populations. Rate constant for aerobic biodegradation of chlorpropham by the sediment derived populations was almost an order of magnitude faster than rate constant for biodegradation by the water-column populations. By varying the sediment/water ratio, the authors also demonstrated that chlorpropham, methoxychlor, and several phthalate esters were less available to biodegradation when sorbed to suspended sediments.

Plate-count estimates of bacterial population densities are very selective and can underestimate total population sizes by three or more orders of magnitude [7,

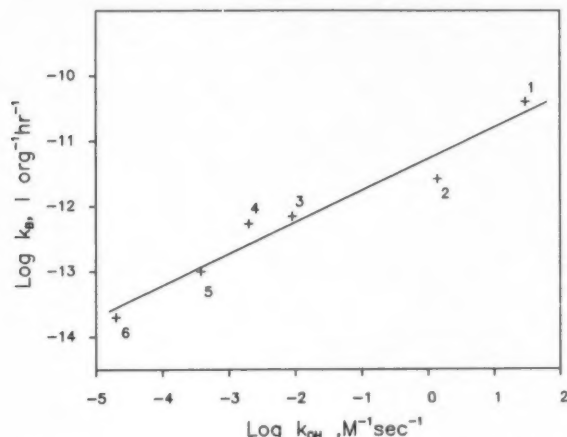


Fig. 3. Correlation of second-order alkaline hydrolysis rate constants (27 °C) determined in distilled water using second-order biolysis rate constants determined in natural water samples. The compounds are 1 butoxyethyl ester of 2,4-D (2,4-dichlorophenoxyacetic acid), 2 malathion, 3 methyl benzoate, 4 methyl anisate, 5 methoxychlor, 6 chlorpropham

8, 27]. Furthermore, although increases in pollutant flux (that is, mg/liter/time or mass degraded/time) with increasing toxicant concentration (consistent with pseudo-first order kinetics) have been observed in nature [19], it is also true that, at elevated xenobiotic concentrations, both a reduction in the apparent first-order rate constant [25] and zero-order kinetics [26] have been observed. The latter phenomena are mathematically consistent with Eq. 5. It is not clear, however, whether these kinetics are a reflection of the maximum growth rate of a specialized sub-population of degraders or are due to toxic effects of elevated concentrations on the microbial community.

Use of Kinetics for Rate Prediction

Development of microbial degradation kinetics has two distinct advantages:

- (1) dependence upon site and time is reduced so application may be made to a broad spectrum of conditions and compounds and
- (2) predictions can be reliably determined for exposure concentration levels.

Second-order kinetics discussed here suggest a possible approach for describing microbial transformation for some organic compounds. This approach involves conditions under which chronic exposure or continuous low input concentrations of compounds into aquatic environment may be assessed. In general, the concentration of the organic compound in natural waters and sediments would be expected to be low and insufficient to support measurable growth of the endogeneous population.

Wolfe et al. [28] established that structure activity relationship may also be a reliable approach to the study of biodegradation of classes of xenobiotics in the

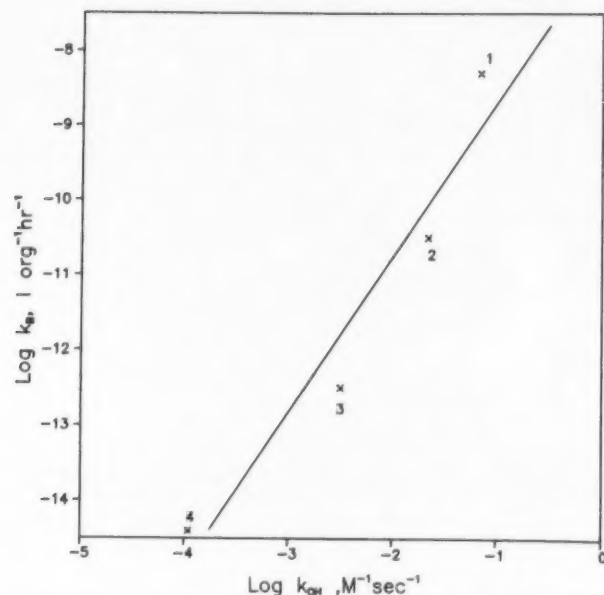


Fig. 4. Correlation of second-order alkaline hydrolysis rate constants ($30^{\circ}C$) obtained in distilled water with second-order biolysis rate constants ($25^{\circ}C$). 1 dimethyl phthalate, 2 di(n-butyl) phthalate, 3 di(n-octyl) phthalate; 4 di(2-ethylhexyl) phthalate

aquatic environment. Good correlations were obtained between second-order microbial degradation rate constants for compounds in natural waters and second-order alkaline hydrolysis rate constants for the same compounds in distilled water (Fig. 3). Likewise, a similar correlation (Fig. 4) for four phthalate esters in sediments was obtained.

Such correlations may serve as alternative or supplemental approaches to the studies of biodegradation of toxic substances. The potential use of these structure-activity relationships seems greatest in the area of exposure assessment modeling when reliable microbial rate constants are not available but may be adequately predicted through structure activity correlations.

Summary

The studies of second-order kinetics cited above are a useful beginning in the detailed quantitative study of rates of biotransformation of xenobiotics. Many questions about the role of temperature, other nutrients, population structure, and compound structure are still unanswered. Only additional kinetic studies for many compounds and sites can provide confidence in the approach and the ultimate answers to these questions.

References

1. Alexander, M.: Recalcitrant molecules, fallible microorganisms, In: *Microbial Ecology* (Lynch, J.M. and Poole, N.J., eds.), Oxford, Blackwell Scientific Publications, 1979, pp. 246-253
2. APHA: Standard Methods for the Examination of Water and Wastewater, 14th ed. Washington, American Public Health Association, 1976, 1193 p
3. Baughman, G.L., Paris, D.F., Steen, W.C.: Quantitative expression of biotransformation rate. In: *Biotransformation and Fate of Chemicals in the Aquatic Environment*. (Maki, A.W., Dickson, K.L., Cairns, J., Jr., eds.), Washington, Amer. Soc. Microbiology, 1980, pp. 105-111
4. Brooks, G.T.: Pesticides in Britain. In: *Environmental Toxicology of Pesticides* (Matsumura, F., Boush, G.M., and Misato, T., eds.), New York, Academic Press, 1972, pp. 61-114
5. Buzzell, J.C., Jr., Thompson, C.H., Ryckman, D.W.: Behavior of Organic Chemicals in the Aquatic Environment. Part III: Behavior in Aerobic Treatment Systems (Activated Sludge), Washington: Manufacturing Chemists Association, 1969, 84 p
6. Conway, R.A., Waggy, G.T.: Amer. Dyestuff Reporter 55(16), 33 (1966)
7. Fletcher, M.: The aquatic environment. In: *Microbial Ecology* (Lynch, J.M. and Poole, N.J., eds.), Oxford, Blackwell Scientific Publications, 1979, pp. 92-224
8. Jannasch, H.W., Jones, G.E.: *Limno Oceanog.* 4, 128 (1959)
9. Lawrence, A., McCarty, P.: J. Sanitary Engng. Div., Proc. Amer. Soc. Civil Engineers. 96 (SA3), 757 (1970)
10. Mateles, R.I., Chian, S.K.: *Environ. Sci. Technol.* 3 (6), 569 (1969)
11. Matsumura, F.: Current pesticide situation in the United States. In: *Environmental Toxicology of Pesticides* (Matsumura, F., Boush, G., Misato, T., eds.), New York, Academic Press, 1972, pp. 33-60
12. Moe, P.G.: *Environ. Sci. Technology* 4 (5), 429 (1970)
13. Monod, J.: *Ann. Rev. Microbiology* 3, 317 (1949)
14. Paris, D.F., et al.: Microbial Degradation and Accumulation of Pesticides in Aquatic Systems. Athens, GA: US Environmental Protection Agency, Rep. EPA-660/3-75-007, 1975
15. Paris, D.F., Lewis, D.L., Wolfe, N.L.: *Environ. Sci. Technol.* 9(2), 135 (1975)
16. Paris, D.F., et al.: Second order model to predict microbial degradation of organic compounds in natural waters. *Appl. Environ. Microbiol.* (In press)
17. Pirt, S.J.: *Principles of Microbe and Cell Cultivation*. Oxford, Blackwell Scientific Publications, 1975
18. Powell, E.O.: *J. Gen. Microbiol.* 15, 492 (1956)
19. Sherrill, T.W., Sayler, G.S.: *Appl. Environ. Microbiol.* 39, 172 (1980)
20. Slater, J.H.: Microbial population and community dynamics. In: *Microbial Ecology*. (Lynch, J.M., and Poole, N.J., eds.), Oxford, Blackwell Scientific Publications, 1979, pp. 45-63
21. Smith, J.H. et al.: Environmental Pathways of Selected Chemicals in Freshwater Systems, Part II, Laboratory Studies. Athens, GA, US Environmental Protection Agency, Report No. EPA/600-7-78-074, 1978
22. Steen, W.C., Paris, D.F., Baughman, G.L.: Effects of sediment sorption on microbial degradation of toxic substances. In: *Contaminants and Sediments*, Vol. 1, Ann Arbor, Ann Arbor Science Publishers, Inc., 1980, pp. 477-482
23. Stumm-Zollinger, E., Harris, R.H.: Kinetics of biologically mediated aerobic oxidation of organic compounds in receiving waters and in waste treatment. In: *Organic Compounds in Aquatic Environments*. (Faust, S.J. Hunter, J.V., eds.), New York, Marcel Dekker, Inc., 1971, pp. 555-598
24. Swisher, R.D.: *Surfactant Biodegradation*, New York, Marcel Dekker, Inc., 1970, 496 p.
25. Tinsley, I.J.: *Chemical Concepts in Pollutant Behavior*, New York-Chichester-Brisbane-Toronto, John Wiley, 1979, p. 265
26. Visser, S.A. et al.: *Appl. Environ. Contamin. Toxicol.* 6, 455 (1977)
27. Wetzel, A.G.: *Limnology*, Philadelphia, W. B. Saunders, Inc., 1975, 743 p.
28. Wolfe, N.L., et al. *Environ. Sci. Technol.* 14(9), 1143 (1980)
29. Wright, R.T., Hobbie, J.E.: *Limnol. and Oceanog.* 10, 22 (1965)